Self-Organization in Living Cells: Networks of Protein Machines and Nonequilibrium Soft Matter

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Abstract. Microscopic self-organization phenomena inside a living cell should not represent merely a reduced copy of self-organization in macroscopic systems. A cell is populated by active protein machines that communicate via small molecules diffusing through the cytoplasm. Mutual synchronization of machine cycles can spontaneously develop in such networks – an effect which is similar to coherent laser generation. On the other hand, an interplay between reactions, diffusion and phase transitions in biological soft matter may lead to the formation of stationary or traveling nonequilibrium nanoscale structures.

Key words: conformational relaxation, diffusion, enzymes, microvolumes, nonequilibrium nanostructures, phase transitions, synchronization

1. Introduction

Living biological cells demonstrate an extremely high degree of internal functional organization and coordination of processes at a molecular level. On the other hand, they are physical systems and must therefore obey the laws of statistical physics. In the middle of the 20th century, E. Schrödinger has shown [1] that the second law of thermodynamics, predicting that entropy and hence the disorder of a physical system should increase with time, is not applicable to open systems in the presence of energy flows. Such systems may maintain and develop their organization in the course of time – a phenomenon which is known as non-equilibrium self-organization. In the last decades, self-organization processes in various physical, chemical and biological systems have been investigated [2–6]. A typical example of chemical self-organization is provided by complex wave patterns spontaneously formed in the Belousov-Zhabotinsky reaction [7, 8]. These patterns are however macroscopic, with a characteristic wavelength of an order of several millimeters.

Some time ago we have asked a question whether self-organization in living cells represents only a reduced copy of spatio-temporal self-organization phenomena in macroscopic reaction-diffusion systems or whether qualitatively new effects become possible at much shorter, microscopic length scales of a single cell [11]. We have noticed [11–13] that the length scales of spatial patterns resulting purely

from an interplay between reactions and diffusion are limited by the diffusion length which can be estimated as $L_{diff} = \sqrt{Dk}$ where D is the diffusion constant and k is the reaction rate. The diffusion length is just the distance passed by diffusively moving molecules between two reactive collisions and, obviously, any developing pattern of chemical concentration should be uniform below this length scale. For typical biochemical reactions $D = 10^{-5} cm^2/s$ and $k = 10^3 s^{-1}$, so that $L_{diff} = 1 \mu$m. Hence, wavelengths of stationary Turing patterns or propagating waves in biochemical systems cannot generally exceed a micrometer.

This conclusion agrees with the experimental data. Rotating spiral waves of Ca^{++} with a wavelength of about 30 μm have been observed inside giant cells (frog eggs) of a millimeter size [9]. Recently, traveling glycolytic waves with the wavelengths of 3 to 4 μm were found in elongated neutropil cells with the size of about 20 μm [10]. But cells can be much smaller (for example, a bacterium *E. coli* has the width of only half a micrometer) and, moreover, a cell may be further divided into submicrometer compartments. Apparently, spatiotemporal pattern formation of reaction-diffusion type cannot account for the high degree of self-organization needed to maintain physiological operation of cells at submicrometer levels [11–13]. Slow uniform biochemical oscillations of chemical concentrations [14] are possible even in such microvolumes. However, their typical temporal periods are in the order of minutes and hours and, while providing a possibility for slow biochemical regulation, they cannot be responsible for rapid microscopic self-organization of intracellular processes.

The functioning of a cell is based on a highly coordinated action of a large population of molecular machines. These machines, representing single proteins or their complexes, are far from thermal equilibrium because they receive energy in the chemical form. This allows them to operate autonomously, overcoming the restrictions set by thermodynamics for equilibrium systems. Active protein machines are immersed into a water solution providing a passive medium needed for the supply of energy and for the communication between the machines. The communication is realized through diffusion of small molecules released by a machine and able to affect the operation of another machine. Small molecules are also employed to submit energy.

For a physicist, this description reminds of the structure and operation of another well-known nonequilibrium system – the solid-state laser. The laser is formed by a population of active atoms immersed into a passive solid-state matrix. Energy is supplied to the atoms with incoherent photons of optical pumping. Interactions between the atoms result from the generation of photons and their action on other atoms (the effect of quantum stimulated emission). When pumping is strong enough, this nonequilibrium system undergoes a spontaneous transition to laser operation characterized by strong correlations in the states of individual active atoms and the coherente of emitted photons [15].

Networks of interacting molecular machines may display a similar behaviour. In a series of publications [16–20] we have shown that mutual synchronization of

turnover cycles can spontaneously develop in populations of reacting enzyme molecules confined to microvolumes. This leads to the development of strong correlations in conformational states of individual protein machines – enzyme molecules, manifested in a coherent release of the product.

Another important physical aspect of a cell is that it contains many subsystems representing weakly condensed matter. Examples of such 'soft' matter are biological membranes, vesicles, the cytoskeleton formed by microtubules, and concentrated polymer solutions. Typically, the organization of soft matter is extremely labile and various phase transitions transforming one equilibrium structure into another are possible. A special feature of the living cell is that its soft matter subsystems are coupled to nonequilibrium chemical reactions. An interplay between phase transitions, reaction and diffusion in weakly condensed matter can give rise to new stationary structures and to traveling waves [21]. In contrast to the patterns based only on reactions and diffusion, the wavelength of nonequilibrium structures in reacting soft matter is not however limited by the diffusion length and may belong to the nanoscale domain. Therefore, they would perfectly fit into the characteristic dimensions of a single biological cell.

These two aspects of microscopic self-organization in living cells are reviewed in the present paper, using examples from our own research. In the next two sections, networks of interacting molecular machines are considered and synchronization phenomena in such networks are analyzed. In the subsequent section, theoretical approaches needed for modeling of nanoscale pattern formation in nonequilibrium soft matter are discussed.

2. Networks of Protein Machines

Diffusion of small molecules in microvolumes is extremely strong. For a small globular volume of linear size L filled with liquid, the mixing time for small molecule with diffusion constant D can be estimated as

$$t_{mix} \approx \frac{L^2}{D}. \tag{1}$$

After this time, a molecule released into the volume will forget its initial position and will be found with equal probability anywhere inside it. For a volume of size $L = 1 \ \mu m$ and the diffusion constant $D = 10^{-5} \text{cm}^2/\text{s}$ typical for small molecules in water solutions, we have $t_{mix} \approx 1$ ms.

Suppose that we have two small molecules of size R that diffuse freely inside a microvolume. After what traffic time $t_{traffic}$ they would collide, meeting each another? A rough estimate for this characteristic time can be obtained using the theory of diffusion-controlled reactions. We find [11–13] that

$$t_{traffic} \approx \frac{L^3}{DR} \tag{2}$$

This is a remarkable result. For particles of nanometer size $R = 1$ nm and for $L = 1$ μm, $D = 10^{-5}$cm^2/s, it predicts that $t_{traffic} = 1$ s. Hence, *any two molecules in a micrometer volume would meet each other every second!* Note that the traffic time depends strongly on the size L of the volume. In submicrometer volumes ($L = 0.1$ μm) it decreases down to a millisecond, whereas in the volumes of size $L = 10$ μm the particles would need a thousand seconds to find each other. Such extreme sensitivity already indicates that very special kinetic regimes may be expected in small cells and inside cellular compartments.

Communication between protein machines is realized by small molecules traveling through the cell volume. When such a molecule arrives at a proper protein, it should dock at a particular site on its surface. The radius of this site would be typically in the nanometer range. The time needed for a messenger molecule to find by simple diffusion a docking site of a given protein can be again estimated using equation (2) where D is then the diffusion constant of small messenger molecules (proteins are heavy and their mobility can be neglected) and R is the radius of the docking site (which is of the same order of magnitude as the size of the messenger molecules). Thus, even if a particular protein machine is present in a single copy inside a cell of a micrometer size, it can be found by a diffusing messenger molecule within a second.

Note that the traffic time is always much larger than the mixing time and their ratio is given by

$$M = \frac{t_{traffic}}{t_{mix}} = \frac{L}{R}. \tag{3}$$

The ratio M has a simple interpretation: a messenger molecule should typically cross M times the reaction volume before it finds a docking site on the surface of a particular single protein. For a cell of a micrometer size, $M = 1000$.

A cell may contain a certain number N of proteins of a particular kind and a diffusively moving messenger molecules can dock to any of them. If N targets are randomly distributed over the volume, the transit time needed to find the first of them and dock to it is estimated as

$$t_{transit} = \frac{1}{N}t_{traffic} = \frac{L^3}{NDR} \tag{4}$$

The ratio

$$M_N = \frac{t_{transit}}{t_{mix}} = \frac{L}{NR} \tag{5}$$

tells us how many times a messenger molecule will cross the entire volume before it finds a target.

Using equation (5), a critical number N_{crit} can be defined as

$$N_{crit} = \frac{L}{R} \tag{6}$$

If a cell contains less than N_{crit} targets of a given kind, the first target will be found by a messenger molecule after crossing at least several times the entire reaction volume, so that the initial location of the messenger molecule is irrelevant and completely forgotten. In the opposite limit of $N \gg N_{crit}$ the first target will be found before the whole volume is crossed, and thus in a neighbourhood of a point where the messenger molecule was released. Note that when $N = N_{crit}$ the transit time coincides with the mixing time, $t_{transit} = t_{mix}$.

The critical concentration c_{crit} of targets can also be defined as

$$c_{crit} = \frac{N_{crit}}{L^3} = \frac{1}{L^2 R} \tag{7}$$

The critical number N_{crit} and the critical concentration c_{crit} of protein targets depend only on the size L of the reaction volume and the radius R of the docking site on a target. For a cell of a micrometer size, $N_{crit} = 1000$ and the critical concentration is $c_{crit} = 10^{15} \text{cm}^{-3} = 10^{-6} M$. In a cellular compartment of size $L = 0.1 \, \mu\text{m}$, we have $N_{crit} = 100$ and the critical concentration increases to $10^{-4} M$.

Depending on the concentration of protein machines of a given kind, different communication regimes can be realized. At high concentrations, communication based on diffusion is local because messengers find their targets in a vicinity of the machine that has released it. On the other hand, when $c < c_{crit}$ the communication is global because any target inside the reaction volume can be found with equal probability, independent of its location and the point where a messenger molecule has started its motion.

Various machines populate a living cell. Some of them act as molecular motors. Other machines are chemical, and their task is to facilitate certain chemical reactions or transitions of ions. A common property of any machine is that it operates out of thermal equilibrium and needs energy for its operation. But energy can be supplied to molecular machines only in discrete portions, with ATP or other small molecules. Each portion of energy provides a kick that pushes a macromolecule to a conformational state far from equilibrium. This is followed by conformational relaxation that tends to return the protein to its equilibrium state.

Generally, the duration of a single cycle of a machine is limited by the time needed for conformational relaxation. Such relaxation is a complex process that involves passing through a sequence of many configurational substates separated by energy barriers. An example of configurational relaxation is yielded by folding of a protein molecule. The relaxation times can differ greatly from one protein to another, they also depend on how far is initially a molecule from its equilibrium conformation.

Many protein machines of a cell are slow, with characteristic cycle times τ ranging from tens of milliseconds to seconds (and even much more for the machines involved in genetic expression). For such machines, dynamical processes of conformational relaxation which are responsible for their operation may be significantly longer than all diffusion times characterizing intramolecular communication.

When the conditions $\tau \gg t_{transit} \gg t_{mixing}$ hold, a population of protein machines effectively represents a *molecular network* where rapid global communication is possible [12, 13]. The network is formed by dynamical elements (protein machines) that interact via exchange of small messenger molecules diffusively traveling through the reaction volume. Since $t_{transit} \gg t_{mixing}$, a messenger molecule released by a given machine may equally probable find any of the machines in the cell, independent of their locations. Moreover, since $\tau \gg t_{transit}$ this would occur within time that is much shorter than the duration of a single dynamical cycle of a machine.

Because of instantaneous long-range interactions, a network of protein machines effectively represents a highly dimensional dynamical system, where internal states of individual machines may become strongly correlated. In other words, coherent collective dynamics of protein machines may develop.

In the last decades, mathematical models of globally coupled periodic [22] and chaotic [23] oscillators have been studied and various synchronization phenomena in such models have been investigated. Synchronization phenomena are possible in populations of neural cells and can play an important role in the brain [24]. Our analysis reveals that populations of protein machines inside a single living cell may form similar networks, where highly coordinated self-organized collective operation takes place.

In the next section we consider an example of a molecular network formed by a population of allosteric enzymes in a microvolume and show that this system can undergo a spontaneous transition to coherent collective behaviour characterized by the synchronization of individual turnover cycles.

3. Mutual Synchronization of Enzymic Turnover Cycles

Enzymes are proteins acting as single-molecule catalysts. Their function is to convert substrates into product molecules, i.e. to realize a reaction $S + E \rightarrow E + P$. A substrate molecule binds to an enzyme and is placed at an active center inside it. The energy barrier for conversion is then significantly reduced, and the substrate is converted into a product and the new product molecule is released into the solution. The enzyme operation is characterized by its turnover rate defined as the number of product molecules released per unit time by a single enzyme molecule, provided the substrate is present in abundance. The inverse of the turnover rate is the turnover time, indicating what time is needed to convert a single substrate molecule. The turnover time may be as short as a microsecond, but typically it ranges from tens of milliseconds to a few seconds.

Are enzymes molecular machines? Not necessarily. Heterogeneous catalysis takes place also in the inorganic matter, e.g. on metal surfaces. It may well be that, once a substrate molecule is bound, it is already correctly positioned in an active center of an enzyme and then should only wait until a fluctuation overcoming a (reduced) energy barrier arrives and transforms a bound substrate molecule into a

product which immediately dissociates. In this case, the role of the protein is only to provide a static equilibrium support with an optimal atomic configuration inside the active center. There is experimental evidence that some enzymes indeed act in this straightforward manner [25]. In this case, conformational transitions are not directly involved in the turnover cycle, though conformational fluctuations (known as dynamic disorder) can still influence the enzyme activity by modulating the height of the energy barrier.

If an enzyme operates as a protein machine, conformational transitions and the processes of conformational relaxation must represent an essential part of its turnover cycle [26]. When a substrate molecule becomes bound to a site on the surface of an enzyme, this may initiate a sequence of conformational changes in the substrate-enzyme complex. After some time, an optimal configuration reducing the electronic energy barrier for the conversion of substrate into product is reached and the conversion occurs. The formed product-enzyme complex may go through another sequence of conformational transitions, bringing the substrate to the surface and facilitating its dissociation. When the product is released, a protein may be found in a conformation different from its equilibrium state and should then return to it. The internal motions of the substrate-enzyme and product-enzyme complexes, as well as the final return of a free enzyme to its equilibrium state, represent different processes of conformational relaxation. The energy kick, bringing a molecule far from equilibrium and initiating the relaxation, is here provided when a substrate molecule is bound. Additional energy may be released when a substrate is converted to product inside the enzyme. A distinguishing feature of enzymes, operating as protein machines, should be therefore that their turnover cycles include many intermediate substates which differ not by their chemical composition, but by their physical configuration corresponding to different conformations of the same substrate-enzyme or product-enzyme complex. The transitions between individual conformational states occur then in an ordered way, as a relaxation process.

Conformational relaxation has been extensively discussed with respect to protein folding, which can be viewed as a process of relaxation from a distant and strongly nonequilibrium initial physical state. Because many intermediate metastable substates, separated by energy barriers of varying height, are present here, the relaxation is best viewed [27] as diffusive drift along a certain conformational coordinate ϕ

$$\frac{d\phi}{dt} = -\Gamma \frac{\partial U}{\partial \phi} + \eta(t) \qquad (8)$$

where $U(x)$ is the free energy landscape, Γ is the relaxation constant and $\eta(t)$ is a white noise of intensity σ, accounting for thermal intramolecular fluctuations. Typically, the energy landscape has the form of a downward slope with small irregular local variations. As a simple model, a linear shape with a constant slope can be chosen. In this approximation, equation (8) is reduced to
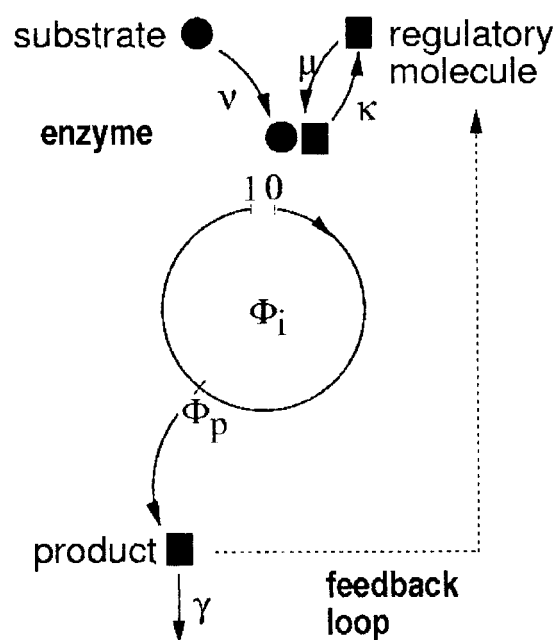
$$\frac{d\phi}{dt} = \upsilon + \eta(t) \qquad (9)$$

Figure 1. Enzyme as a protein machine.

which describes a diffusive drift at a constant mean vetocity υ accompanied by random fluctuations.

Applying such concepts to the operation of an enzyme machine, its turnover cycle is modelled as diffusive drift motion along a conformational coordinate ϕ which can be considered as the cycle phase (Figure 1). The cycle begins with binding of a substrate molecule. The first conformational substate of the substrate-enzyme complex corresponds to the phase $\phi = 0$. Starting from this point, the enzyme begins its internal relaxational motion described by equation (9). When the phase $\phi = \phi_p$ inside the cycle is reached, a product molecule is released. This is followed by the relaxation of the free enzyme to its equilibrium conformation. For simplicity, we assume that this process is again described by equation (9) and corresponds to the phase interval from $\phi = \phi_p$ to $\phi = 1$. In this model, the mean duration of a turnover cycle is $\tau_0 = < \tau > = 1/\upsilon$ and the relative statistical dispersion of turnover times is $\xi = (1/\tau_0)(< \Delta\tau^2 >)^{1/2} \approx (2\sigma\tau_0)^{1/2}$.

The activity of enzymes can be allosterically regulated. Allosteric enzymes have an additional site where small regulatory molecules can bind. Binding of a regulatory molecule induces a transition of the enzyme to a different conformation, where the binding probability rate for a substrate molecule is modified. It is increased in the case of allosteric activation and decreased for allosteric inhibition. The regulatory molecules often represent products of the same enzymic reaction, so that product activation or product inhibition takes place.

As an example, we consider a simple reaction scheme

$$S + E \rightarrow E + P, P \rightarrow 0 \tag{10}$$

where binding of the substrate S is allosterically activated by the product P. The substrate concentration is maintained constant and the product is continuously degraded to prevent its accumulation. The decay rate of molecules is higher than the enzymatic turnover rate. An individual turnover cycle includes a sequence of transitions through different conformational substates, as explained above. The reaction proceeds in a microvolume containing a relatively small number of enzyme molecules. For this reaction, the conditions of a molecular network are satisfied, that is $\tau_0 \gg t_{transit} \gg t_{mixing}$.

Direct stochastic simulations of the reaction (10) have been performed [18]. In these simulations, the population of enzymes consisted of $N = 200$ molecules and the intensity of allosteric regulation was varied (the strength of allosteric activation can be specified by the ratio r of substrate binding rates in the presence and in absence of a bound regulatory molecule). Binding of substrate and decay of product molecules were modelled as discrete probabilistic events; the internal conformational dynamics of individual enzymes was modelled as a stochastic process described by equation (9).

When allosteric regulation was weak ($r = 1.2$), incoherent kinetics has been observed (Figure 2). In this classical kinetic regime, the turnover cycles of individual enzymes are not correlated. The histogram of the distribution over cycle phases ϕ at a given time shows (Figure 2a) that the phases are uniformly distributed along the entire interval from 0 to 1 (a peak at $\phi = 0$ corresponds to enzymes in the ground state waiting to bind a substrate). The number m of product molecules in the reaction volume fluctuates around a certain mean level (Figure 2b) and a Poissonian distribution for this number is found (Figure 2c).

Increasing the strength of allosteric regulation to $r = 2$ changes drastically the reaction kinetics. Now enzymes form two coherent groups (Figure 3a). Inside a group, the phases are strongly correlated and there is a phase shift of half a turnover time between the groups. This synchronous behaviour is manifested in rapid periodic spiking in the number of product molecules at a period equal to the half of a turnover time (Figure 3b). Because of such spiking, the statistical distribution for these molecules is strongly non-Poissonian (Figure 3c).

It should be emphasized that rapid spiking, resulting from synchronization of intramolecular dynamica in individual enzyme molecules, is qualitatively different from slow kinetic oscillations (which are not possible for the considered reaction (10)). In kinetic oscillations, the distributions of reactants remain Poissonian with their mean numbers slowly varying with time. In contrast to this, the synchronous kinetic regimes are characterized by strong deviations from equilibrium in the statistical distributions of reactants already at a microscopic level.

To illustrate the differente, an example of optical systems can be discussed. First, we take a lamp and incorporate it into an oscillatory electric circuit. As a
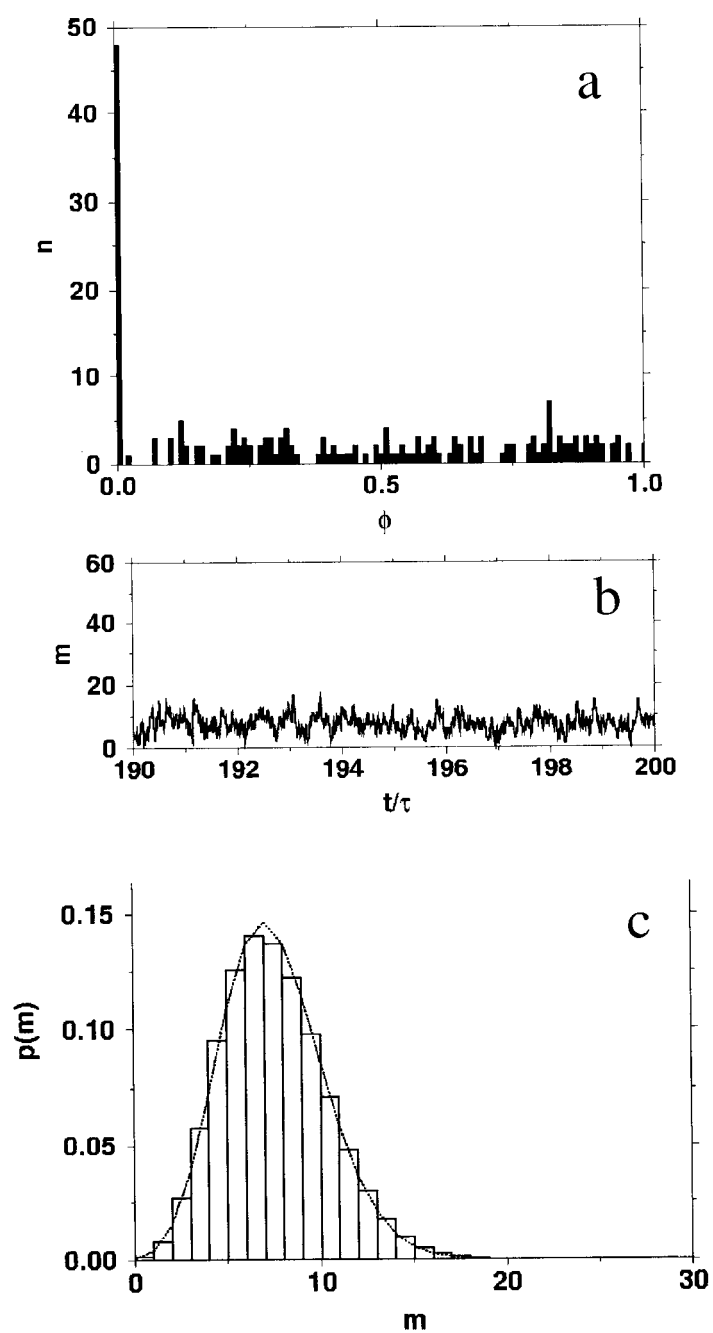
Figure 2. Incoherent kinetic regime in a population of 200 enzyme molecules: (a) distribution over cycle phases, (b) time dependence of the number of product molecules, and (c) statistical distribution of the number of product molecules in the microvolume. From [18].

Figure 3. Coherent kinetics in a population of 200 enzyme molecules: (a) distribution over cycle phases, (b) time dependence of the number of product molecules, and (c) statistical distribution of the number of product molecules in the microvolume. From [18].
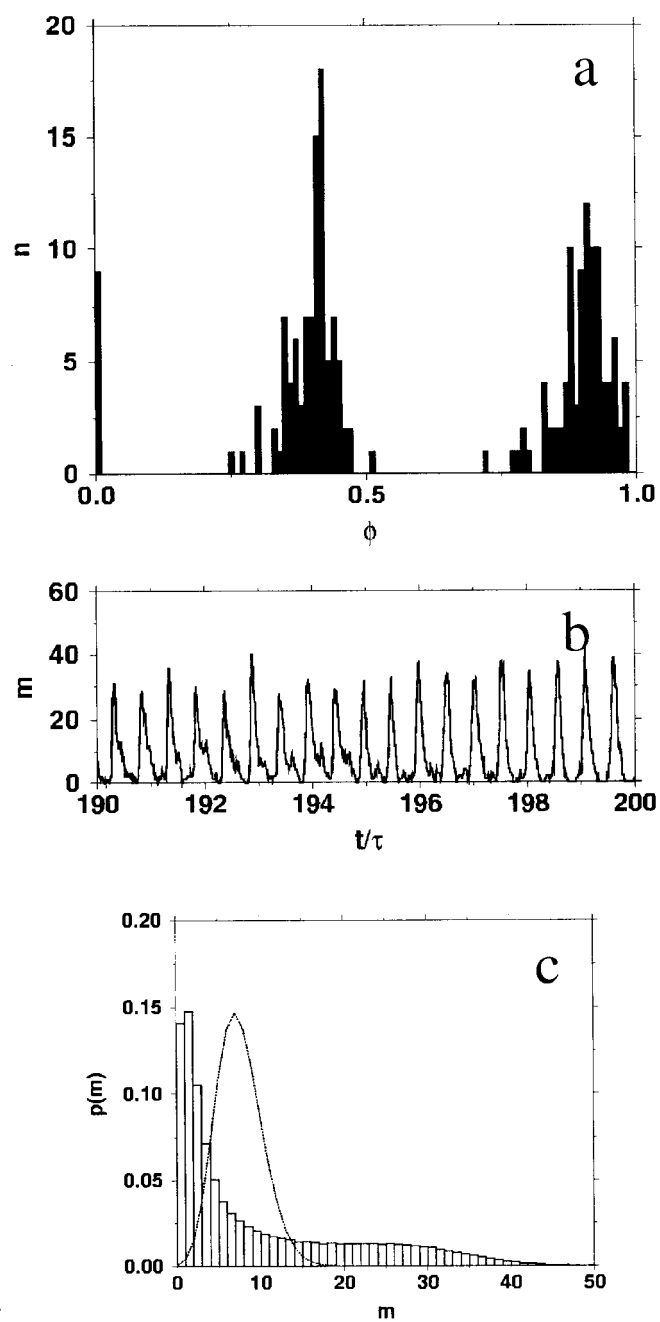
result, the intensity of the emitted light will be periodically varying with time. However, such intensity oscillations are still incoherent, and the statistics of emitted photons remains Poissonian here. On the other hand, if we take a laser we shall find that its operation is coherent. This means that the photon statistics is strongly non-Poissonian and the internal states of active atoms are rigidly correlated. The deviations from thermal equilibrium are observed then already at a microscopic level, for the probability distributions of individual atoms.

The synchronous kinetic regimes of molecular networks represent classical analogs of the quantum laser operation. In the last years, systematic theoretical investigations of coherent collective kinetics for various models of allosteric reactions with product activation [16–18] and product inhibition [19] have been performed. Similar behaviour was also found for non-allosteric enzymic reactions with substrate loops [20]. The results of these studies indicate that mutual synchronization of molecular turnover cycles is a robust phenomenon, which is found under various model modifications. Importantly, the synchronization persists even for relatively high intensities of intramolecular fluctuations when the statistical dispersion of turnover times reaches 20%.

Experimental observations of spontaneous coherent activity in enzymic populations confined to microvolumes are not yet available. However, interesting experiments with external synchronization of enzymic turnover cycles have been performed. In these experiments [28–30] with the photosensitive cytochrome P-450 dependent monooxygenase system the turnover cycles of individual enzymes ware synchronized by applying repeated light flashes at a period $T = 1.32$ s which was only slightly shorter than the turnover time of these enzymes. Then periodic illumination was stopped and the time dependence of the product concentration was optically monitored. It was found that the product concentration changes in steps, with the duration of each single step close to the turnover time of an enzyme molecule. Within a step, the product concentration remains constant because all enzymes are inside their cycles and are therefore 'silent'. At the end of a step, the product concentration is steeply increased because many enzymes have synchronously released product molecules. Several such steps, following initial optical synchronization, have been observed. They become less pronounced and finally fade away with time because of the presence of intramolecular fluctuations. From the experimental data, the statistical dispersion of turnover times of about 20% has been deduced [30].

Though only the effects of mutual synchronization in molecular enzymic networks have been discussed here, similar behaviour can be expected in networks of other molecular machines populating a living cell. The biochemical activity of a cell can be compared with operation of large industrial factory where certain parts are produced by a system of machines. Products of one machine are then used by other machines for manufacturing of their products or for regulation of their functions. Two possible modes of operation of such a factory can be imagined. In the asynchronous mode, the parts produced by all machines are first depos-

ited and accumulated in a common store. They are taken back from this store by other machines, when the parts are needed for further production. This kind of organization is not however optimal, since it requires large storage facilities and many transactions. It becomes deficient when intermediate products are potentially unstable and can easily be lost or damaged during the storage process. When the synchronous operation mode is employed, the intermediate products, required for a certain operation step in a given machine, are released by other machines and become available exactly at the moment when they are needed. Hence, large storage facilities are eliminated and the entire process may run much faster. Our analysis suggests that under certain conditions the biochemical subsystems of a cell may operate in the synchronous mode. When this occurs, the entire population of reacting and interacting protein molecules can be viewed as a highly connected coherent dynamic molecular network.

The characteristic feature of synchronization phenomena in molecular networks is that complex self-organized structures develop not in the physical, coordinate space, but in the 'internal' space of a population of protein machines corresponding to their various conformations.

4. Self-Organization in Reactive Soft Matter

As already mentioned, spatiotemporal pattern formation based purely on reactions and diffusion is impossible on the scales shorter than the characteristic diffusion length which is of order of a micrometer for typical biochemical reactions. In contrast to this, equilibrium structures in condensed systems with attractive physical interactions between particles can be very small. Atomic clusters may include just tens or hundreds of atoms. 'Quantum dots' on specially prepared metal surfaces have the characteristic size of a few nanometers. Generally, the minimum limit for the size of equilibrium structures is determined by the radius of attractive interactions between molecules in such systems, which can be a fraction of a nanometer. To obtain nonequilibrium nanoscale structures, systems with both chemical reactions and physical interactions between the particles must be considered. These structures may result from the joint action of cohesion, reactions and diffusion. The cohesion, responsible for the formation of equilibrium thermodynamics structures, should not however be too strong. Since the reaction effects are relatively weak, they can compete with physical interactions between the particles and thus influence the microscopic organization of a system only if it is structurally labile.

Structural lability is a feature of 'soft matter' representing molecular systems with weak attractive interactions. The examples of such soft matter are provided by liquid crystals, Langmuir films, lipid membranes, vesicles, or polymers. These condensed systems often have a great number of different equilibrium structures and can easily undergo phase transitions when the medium parameters are changed. Stable nonequilibrium structures emerge from an interplay between such structural phase transitions and chemical reactions, coupled to diffusion [21].

Suppose that a system represents a mixture of molecules A and B, such that the molecules of the same kind attract each another and there are repulsive interactions between molecules A and B. If the repulsion is sufficiently strong, this mixture undergoes phase separation and spatial domains filled predominantly by particles A or B are formed. At the initial stage of the phase separation process, the domains are very small. In the course of time their sizes are however increasing until eventually the whole medium becomes divided into just two domains occupied by particles A and B. The kinetics of this process, which can be viewed as complex relation to an equilibrium spatially segregated distribution, is controlled by diffusion of particles and interactions between them.

Let us add now the reactions $A \leftrightarrow B$ which convert different kinds of molecules one into another. If the system is closed and the reactions result only from thermal activation, they cannot influence the equilibrium state of the system determined by the minimum of its free energy (indeed, kinetic coefficients such as the diffusion or reaction rate constants cannot enter in an expression for free energy). Therefore, though the kinetics of the relaxation process may be different, the asymptotic state of the system should be the same as in absence of the reactions, i.e. the medium will still separate into macroscopic phase domains.

The behaviour becomes qualitatively different when the system is open and energy is supplied to it. Suppose, for example, that the first reaction is photoinduced, i.e. we have $A + \bar{h}\nu \rightarrow B$. Now energy is supplied with the flux of photons and then dissipated into a thermal bath which should be connected to the system to prevent its heating. When the energy flow is strong enough, the system is far from thermal equilibrium and its asymptotic state should no longer correspond to a minimum of free energy. Theoretical investigations [31–33] show that, instead of the macroscopic phase separation, this system develops a periodic stationary spatial pattern of domains filled with particles A or B. The wavelength of this pattern can be roughly estimated [33] as

$$\lambda \approx \sqrt{r_0 L_{diff}} \tag{11}$$

where r_0 is the characteristic interaction radius and L_{diff} is the diffusion length defined as $L_{diff} = \sqrt{D/k}$ where k is the rate constant of the photoinduced chemical reaction. It is determined by the rate of the chemical reaction and, therefore, by the rate of supply of energy to the system. We see that $\lambda \propto k^{-1/4}$ and thus the wavelength goes to infinity as the reaction rate is decreased. This effect of nonequilibrium pattern formation has been experimentally studied [34] for polymer blends in the presence of photoinduced reactions. The predicted dependence of the wavelength on the illumination intensity has been confirmed.

Analyzing the above expression for λ, we can notice that, because the interaction radius r_0 is just a few Å and much shorter than the diffusion length L_{diff}, the wavelength should lie in the range $L_{diff} \gg \lambda \gg r_0$. Hence, nonequilibrium stationary structures with spatial scales shorter than the diffusion length can be obtained. Such dissipative structures exist because of the energy flow acting on the

system. Their properties can be directly controlled by varying the rate of energy supply.

In contrast to equilibrium systems where only stationary structures may be observed, nonequilibrium physical systems can also show regular or chaotic self-oscillations and may support active propagation of waves. Many different traveling wave patterns are known in the theory of reaction-diffusion systems. However, the smallest characteristic wavelength of such traveling patterns is again limited by the diffusion length and cannot be very short. Such a limitation is absent for systems combining reactions and diffusion with potential energetic interactions between particles. Traveling nanostructures, that contain as small as only hundreds of reading atoms, are possible under these conditions.

Hildebrand et al. considered [35] a system with surface chemical reactions in the presence of interactions between adsorbed molecules. Their model can be interpreted as involving three different species A, B and C (the species A and B correspond to adsorbed molecules and the species C are vacant lattice sites). The surface reaction scheme is

$$C \leftrightarrow A, C \to B, A + B \to 2C + P \qquad (12)$$

where the first two steps represent adsorption and desorption of molecules A and B from the gas phase; the last step describes a reaction between A and B leading to the formation of two vacant sites C and a product molecule P that immediately leaves the reactive surface. It was assumed that attractive lateral interactions between molecules A and also between molecules A and B were present. The reaction is operated under flow conditions, where molecules A and B are continuously supplied to the gas phase and the reaction product is pumped away. The theoretical analysis and numerical simulations have shown [35] the existence of periodic traveling waves of chemical concentrations in this model. The waves spontaneously emerge starting form an unstable uniform state. Their wavelength λ can be again roughly estimated by equation (11) where k is the rate constant of the annihilation reaction. Thus, it can also be much shorter than the diffusion length and lie in the nanoscale range.

Because the characteristic sizes of such traveling structures are very small, fluctuations must be taken into account. Stochastic simulations (see [35, 36]) show that the fluctuations break periodic traveling waves into small irregular fragments. Figure 4 shows an example of such traveling wave fragments. Here the total size of the system is only 555 elementary lattice lengths l_0, the interaction radius is $r_0 = 9l_0$ and the diffusion length is $Ldif = 327l_0$. Hence, each fragment contains only about a hundred of molecules. In the course of time, the fragments change their shapes, split or merge. Nonetheless, the propagation remains robust and the velocities of different fragments are close (this is clearly seen examining the space-time cross section in Figure 4). Because of the physical cohesive interactions between molecules, propagation of waves in this model involves mass transport and therefore the fragments essentially represent traveling molecular clusters.
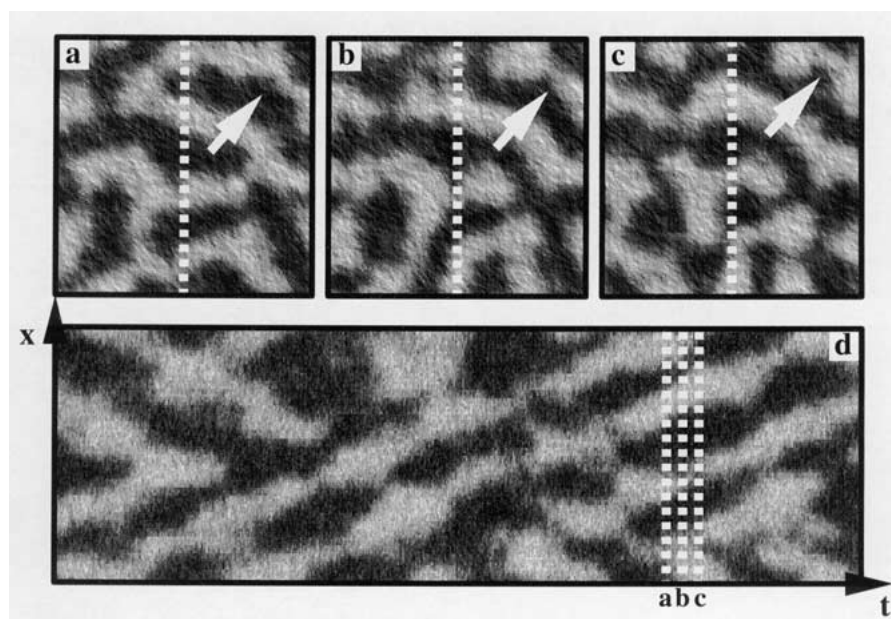
Figure 4. Traveling nanoscale structures. Three snapshots (a, b, c) and the space-time diagram corresponding to the central vertical cross section. From [35].

Recently, Okuzono and Ohta have studied [37] a model of a three-component phase-separating mixture with a cyclic reaction scheme

$$A \rightarrow B \rightarrow C \rightarrow A \tag{13}$$

where strong repulsive interactions between molecules *A* and *B* were present. The solutions in the form of traveling lamellar and hexagonal structures have been found here.

Traveling waves in soft matter have been observed in the experiments with illuminated Langmuir films [38], though a satisfactory theory of this effect is not yet available. In this case the characteristic wavelength of a pattern was still relatively large, lying in the range of tens of micrometers. Traveling 'atomic strings' with the sizes of less than a micrometer have been seen using atomic force microscopy (AFM) on crystal surfaces under reaction conditions [39].

Another effect of self-organization in reactive soft matter is the formation of dissipative localized nanostructures. Such structures were found in a theoretical study of surface chemical reactions [40]. Here, adsorption of particles on a metal surface induces a phase transition changing the arrangement of atoms in the top metal layer. In return, surface regions with the modified structure become strongly attractive for the adsorbed diffusing particles and they aggregate their. In absence of a reaction, such modified regions would grow until they spread over the entire surface bringing it to a different structural state. When a reaction is present, it removes molecules from such regions and thus acts against the diffusive flow supplying the

molecules into them. These two effects can balance each other, and stable circular regions with the modified surface structure and a high concentration of adsorbed molecules are produced. The sizes of these regions may be much smaller than the diffusion length. Because the reaction predominantly proceeds inside such small regions, where adsorbed molecules are concentrated, they can be viewed as self-organized nanoscale microreactors. In contrast to equilibrium static nanostructures, such as 'quantum dots', self-organized nanoreactors exist only in the presence of a reaction. Their site and stability can be controlled by varying the reaction rate. Moreover, they are not pinned to the surface and, by applying a gradient of a chemical concentration, one can move them over the surface. Remarkably, mathematical models used to describe such nanoscale self-organization phenomena bear strong analogies to the equations describing the formation of bacterial colonies [41].

Though nanoscale self-organization in soft matter has so far been mainly theoretically investigated for polymer blends and catalytic surface reactions, such effects are possible and should play an important role in other kinds of soft matter typical for the living cells. Membranes consisting of two kinds of lipid molecules undergo phase separation, which leads under equilibrium conditions to the segregation of components, deformation of the membrane, and budding of a vesicle. When a reaction transforming one kind of molecules into another is added, this may lead to the stabilization of a spatially modulated distribution and complex stationary membrane shapes. If a biomembrane includes active proteins, the spectrum of possible behaviours is even more rich. Besides of stationary periodic or localized structures, oscillations and traveling waves become possible. This means that a biomembrane would itself act like a micromachine. Systematic investigations of self-organization phenomena in condensed systems of a living cell are a challenge for future research.

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